Imagen ® SYBR Green RT-qPCR Premix

Cat. NO IPQ

Description

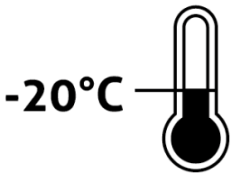
Imagen ® SYBR Green RT-qPCR Premix provide user a rapid and simple way to quantify the expression of target mRNA based on Real-time PCR system containing SYBR Green in just a single tube. Yeastern's Deoxy+ HiSpec RT, Hotstart DNA polymerase and all the components for Real-time SYBR Green RT-PCR are skillfully mixed within a single tube. Unique buffer system allows highly specific quantification in real-time RT-PCR by preventing the formation of nonspecific products and primer–dimers.

Contents

The **Imagen ® SYBR Green RT-qPCR Premix** is supplied as a ready-to-use 2x reaction mix. The formulation contains Hotstart Taq DNA polymerase, Reverse transcriptases, $MgCl_2$, dNTPs, SYBR Green I, reaction enhancers, and stabilizers.

Storage

- ✓ -20 °C
- ✓ Protected from light
- ✓ Avoid repeated freezing and throwing



Reaction Mix Thawing and Handling

Imagen ® SYBR Green RT-qPCR Premix is delivered in a 2x ready-to-use format. To use the mix, thaw the vial on ice to 4 °C.

Please completely mix the vial and briefly centrifuge to ensure all components are at the bottom of the tube. Store on ice protected from light until ready to use. If using automated liquid handling, let sit at ambient temperature for 10 min to further reduce the viscosity.

Application

- ✓ Detection and Quantification of RNA targets.
- ✓ High Throughput Applications.

Prepare the qPCR Reaction Mix

1. Mix the **Imagen ® SYBR Green RT-qPCR Premix** thoroughly but gently until it's completely
2. homogenous.
   Prepare the qPCR Reaction Mix for the number of reactions required as shown in table below and plus 10% overage.

Reagent	Volume (ul)	Final conc.
Imagen ® SYBR Green RT-qPCR Premix	12.5	1x
Forward Primer(10 uM)	0.75	300 - 600 nM
Reverse Primer(10 uM)	0.75	300 - 600 nM
RNA Template	2	100 ng - 10 pg
Nuclease-free water	9	-
Final volume	**25**	-

3. Vortex the tube to mix the contents thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube. (*Use good pipetting practice to ensure assay precision and accuracy of dispensing.)
4. Add DNA (and nuclease-free water, if needed) to the PCR tubes or wells containing the reaction mix, seal tubes or wells with flat caps or optically transparent film, and gently vortex to ensure thorough mixing of the reaction components.
5. Program the thermal cycling protocol on the real-time PCR instrument.

Step		Temp. °C	Time	Cycles
Reverse Transcription		48°C	30 min	1
DNA polymerase activation and template denaturation Amplification		95°C	10 min	1
Amplification	Template denaturation	95°C	20 sec	35-40
	Annealing / Extension and plate read	58 - 65°C	≧40 sec © Data acquisition	
Melt Curve		95°C 60°C 95°C	20 sec 20 sec 15 sec	1

6. Load the PCR tubes or plates onto the real-time PCR instrument and start the qPCR run program.
7. When thermal cycling is complete, perform data according to the instructions in the instrument-specific software.